

QTL Mapping – An Important Technique for Genetic Mapping

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ABSTRACT

Quantitative Trait Loci (QTL) mapping is considered as a standard procedure for mapping of quantitative traits in quantitative genetics in these days. With the help of QTL mapping, one can determine the relative position of a gene on chromosome or plasmid and an association between phenotype and genotype of markers. Choice of mapping population, used for mapping, depends on many factors as plant species, type of marker system used and the trait to be mapped. Among many mapping populations, Backcross and F₂ populations are the simplest to use. Single-marker analysis, interval mapping by maximum likelihood, interval mapping by regression and composite interval mapping are some of widely used methods for detecting QTL.

Key words: QTL, Genetic mapping, Mapping populations, Molecular marker

INTRODUCTION

There are various applications of molecular markers in a species, in which genetic mapping is also important. It refers to determination of relative positions and distance between genes on a DNA molecule (chromosome or plasmid). In this, the positions and relative genetic distances between markers along chromosomes are determined, which is analogous to signs or landmarks along a highway where the genes are “houses”^{1,2}. T.H. Morgan and his student, Alfred Sturtevant published first genetic map in which they showed the locations of six sex linked genes on the chromosome of fruit fly. So, the main aim of genetic mapping is to

study the expression and regulation of genes in which construction of detailed genetic maps with high levels of genome coverage is a first step for some of the applications of molecular markers in plant breeding³.

Some of the main purposes of genetic mapping include detailed genetic analysis of qualitative and quantitative traits which is helpful in genes or quantitative trait loci (QTL) localization^{4,5,6}. It is helpful in desirable genes or QTLs introgression with the help of marker-assisted selection. The evaluation of similarity between genes orders and function in the expression of a phenotype is done with comparative mapping between different species^{7,8}.

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On the basis of chromosome translocations, DNA sequence or other direct measures, it provides a framework for anchoring with physical maps⁶. For economically important traits, positional or map based cloning of genes is done and for this, genetic mapping constitute the first step^{4,9}.

For all these purposes, a genetic linkage map should follow some methodological and technical criteria such as simplicity, robustness, transferability, speed and cost effectiveness¹⁰. This article reviews two important techniques for linkage mapping as a prerequisite for plant improvement programs i.e. linkage analysis and quantitative trait loci.

Quantitative Trait Loci (QTL) mapping:-

QTL mapping may be defined as statistical analysis of the alleles (that occur in a locus) and phenotypes (physical form of traits that they produce). QTL mapping may be defined as process of constructing linkage maps and conducting QTL analysis to identify traits associated genomic regions¹¹. The QTL studies done up to date indicate an L-shaped distribution of QTL effects (i.e. most QTLs show small effect and only a few have strong effect), this enable the identification of QTLs with a major effect on phenotype¹². The first and most basic step in QTL mapping is collection of genotypic (based on molecular markers) and phenotypic data from a segregating population and then statistical analysis is done to reveal all possible marker loci where allelic variation correlates with phenotype. As this procedure only allows for an approximate mapping of QTL, this is generally refers to as primary QTL mapping¹³.

Principle of QTL Analysis:

The main principle behind QTL analysis is detection of an association between phenotype and genotype of markers. On the basis of presence or absence of a particular marker locus, mapping population is partition into different genotypic groups using markers and determining whether significant differences exists between groups with respect to the trait being measured^{14,15}. If there is significant difference between phenotypic means of the

groups (either 2 or 3), it indicates that the marker locus which is used to partition the mapping population is linked to a QTL controlling the trait, depending on the marker system and type of population.

Steps in QTL mapping¹⁶

The general steps involved in a traditional QTL mapping experiment are as follow. First of all, selection of two parental strains is done which have variation for alleles that affect variation in a trait, as different allelic combination can yield the same phenotypic mean. Then, selected parents are crossed to develop an appropriate mapping population. After that, phenotyping of mapping population for the trait(s) of interest (morphological characters, agronomic traits, disease and pest scores etc.) is done under the conditions of greenhouse, screen-house and field. By using adequate number of uniformly spaced polymorphic markers, molecular data on the population is developed and genetic map is constructed. By using statistical programs, molecular markers linked to the trait(s) are identified. These steps are explained in detail stepwise below:

Mapping populations used in QTL mapping experiments: Heterozygous F₁ hybrids are used for the production of various types of mapping population:

- a) **Double haploid lines (DHLs):-** Pollen (haploid) of the F₁ plants are used for regeneration and treated with colchicine to produce double haploid homozygous plants in which every locus is homozygous. Here, DHLs are representing a direct sample of the segregating gametes as pollen population has been generated by meiosis.
- b) **Backcross (BC) population:-** In this, F₁ plants are backcrossed to one of the parents.
- c) **F₂ population:-** It is produced by selfing of F₁ plants.
- d) **Recombinant inbred lines (RILs):-** Individual F₂ plants are selfed and then single seed descent method is used for derivation of inbred generation.

Choice of the type of mapping population depends on many factors such as type of marker system used, plant type and the trait which is to be mapped later on as each of mapping populations mentioned above possesses many advantages and disadvantages. For self-pollinated species, simplest types of mapping populations developed are F_2 populations and BC populations.

Construction of Genetic/Linkage maps: A linkage map may be defined as a 'road map' of chromosomes which is derived from two different parents. Position and relative genetic distances between markers along chromosomes can be indicated by linkage maps. Genotyping data generated on any of the above mentioned mapping populations is an important step for construction of a linkage map before initiating any QTL analysis. There is a mixture of parental and recombinant genotypes in a segregating population. To infer genetic distance between markers, calculation of recombination fractions is done with the use of frequency of recombinant genotypes. Segregation of markers is analyzed to find out the relative order and distances between markers; lower frequency of recombination between two markers indicates that they situated closer to each other and vice-versa. Linkage between markers is usually calculated with an odds ratio (i.e. ratio of linkage versus no linkage). It is also called a logarithm of odds (LOD) value or LOD score¹⁷. For construction of linkage maps, LOD values of >3 are typically used². Linked markers are grouped together into linkage groups which represent chromosomal segment or can represent entire chromosome. Software programs most commonly used for construction of linkage maps include Mapmaker/EXP^{18,19}, MapManager QTX²⁰, and THREaD Mapper Studio²¹. and they are freely available from the internet. Another commonly used program for construction of linkage maps is JoinMap²².

Detection of QTLs: For detection of QTLs, four most widely used methods are single-marker analysis, interval mapping by maximum likelihood, interval mapping by regression and composite interval mapping.

- a) **Single-Marker Analysis:-** It is a traditional method to detect QTL. When comparing phenotypic means for progeny of each marker class (e.g., means of the marker classes AA, Aa, aa), difference between two means provides an estimate of the phenotypic effect of substituting an 'A' allele by an 'a' allele at the QTL. A simple statistical test, such as t-test or F-test is used to test whether the inferred phenotypic effect is significantly different from zero. A significant value is indicating that a QTL is located in the vicinity of the marker.
- b) **Interval Mapping by Maximum Likelihood:-** It is the most commonly used method of QTL analysis. The basic principle behind interval mapping is to test a model for the presence of a QTL at many positions between two mapped marker loci. This model is a fit and the method of maximum likelihood is used to test its goodness. Models are evaluated by computing the likelihood of the observed distributions with and without fitting a QTL effect.
- c) **Interval Mapping by Regression:-** It is simplification of the maximum likelihood method²³. It is essentially same as the method of basic QTL analysis (regression on coded marker genotypes) except that phenotypes are regressed on QTL genotypes. QTL genotypes are replaced by probabilities estimated from the nearest flanking markers as QTL genotypes are unknown.
- d) **Composite Interval Mapping:-** Fitting the model for a QTL at only one location is one of the major limiting factor of interval mapping. There are two problems with this approach: (a) the effects of additional QTL will contribute to sampling variance and (b) if two QTLs are linked, their combined effects will cause biased estimates. For this, composite interval mapping was proposed as solution^{24,25,26}. This method gives more power and precision than simple interval mapping because the effects of the other QTLs are

not present as residual variance. This method can remove the bias that can be caused by QTLs that are linked to the position being tested.

QTL mapping Softwares: There are over 100 genetic analysis software packages (linkage analysis and QTL mapping), some of most commonly used software packages are as follow with some of features:

1. MapMaker/QTL (<ftp://genome.wi.mit.edu/pub/mapmaker3/>): It is a user-friendly, freely distributed software program runs on almost all platforms. By using standard interval mapping, it analyzes F_2 or backcross data.
2. MQTL: MQTL is a computer program for CIM in multiple environments and it can also perform SIM. Currently, MQTL is restricted to the analysis of data from homozygous progeny (double haploids, or RILs) and progeny types with more than two marker classes (e.g., F_2) are generally not handled.
3. PLABQTL (<http://www.uni-hohenheim.de/ipspwww/soft.html>): PLABQTL is a freely distributed, most commonly used computer program for SIM and CIM of QTL. Its main purpose is to localize and characterize QTL in mapping populations which is derived from a biparental cross by selfing or production of double haploids. Currently, this is the easiest software for composite interval mapping.
4. QTL Cartographer (<http://statgen.mcsu.edu/qtlcart/cartographer.html>): QTL Cartographer is QTL software mainly written for UNIX, Macintosh, or Windows. It performs single-marker regression and composite interval mapping. It permits analysis from F_2 or backcross populations. It displays map positions of QTLs using the GNUMPLOT software.
5. MapQTL (<http://www.cpro.dlo.nl/cbw/>): MapQTL is a licensed software program. It performs Kruskal–Wallis test (single-marker analysis), CIM and multiple interval mapping on almost all kinds of mapping populations.

6. Qgene: Qgene is a QTL mapping and marker-aided breeding package written for Macintosh. It has a user-friendly graphical interface and produces graphical outputs. QTL mapping is conducted by either single-marker regression or interval regression.

7. SAS: SAS is general statistical analysis software. It can detect QTL by identifying associations between marker genotype and quantitative trait phenotype by single-marker analysis approach such as ANOVA, t-test, GLM, or REG.

Importance of QTL mapping

As we know that majority of the economically important characters are inherited quantitatively and controlled by a large number of genes called polygenes whose exact number, location and mode of action is difficult to be found through Mendelian analysis. The association of this analysis can provide evidence for the genetic control of traits variation but is not very precise because the genetic effects associated with marker genotypes are confounded by the position of a functional QTL and its actual effect. If markers are so highly dense that they are generated at QTL positions, a simple marker-phenotype association analysis may be useful. The generation of such high-density maps is not possible for a majority of species in practice. Powerful analytical techniques are needed to separate the effects of a QTL from its location.

Unlike molecular markers, the genomic locations of QTLs are unknown and should be inferred on the basis of the association analysis of the phenotypes and markers. The role of statistical methods is in the identification, mapping and estimation of functional QTLs using location known, neutral markers. One of the most important statistical foundations for QTL mapping is laid out in the mixture different quantitative models, in which each observation is assumed to have arisen from one of unobservable QTL genotype groups, each group being suitably modeled by a density from some parametric family. This model provides a framework by which

observations may be clustered together into genotype groups for discrimination.

CONCLUSION

Quantitative traits are controlled by polygenes and the regions within genomes that contain genes associated with a particular quantitative trait are known as Quantitative Trait Loci (QTLs). The identification of QTLs based on conventional evaluation is not possible. Therefore, the uses of DNA markers with construction of linkage maps for diverse crop species are vital to identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis. Application of linkage maps and QTL analysis are important tool to identify genomic regions associated with traits. The DNA markers that are tightly linked to agronomically important genes may be used as molecular tools for Marker-Assisted Selection (MAS) in plant breeding. Despite optimism about continued yield improvement from conventional breeding, new technologies such as DNA marker technology will be needed to maximize the probability of success.

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